

CLAIMS

What is claimed is:

- 5 1. An antibody microarray screen comprising: a substrate; monoclonal and polyclonal antibodies that are purified immunoglobins, wherein said antibodies are spotted on predetermined positions on said substrate; and fluids unprocessed for immunoglobulin isolation, wherein said unprocessed
10 fluids are spotted on said predetermined positions on said substrate.
2. The antibody microarray screen according to claim 1, wherein said antibodies detect proteins selected from the group consisting of drug-metabolizing enzymes and proteins functionally related with said drug-metabolizing enzymes.
- 15 3. The antibody microarray screen according to claim 2, wherein said drug metabolizing enzyme is cytochromes P450.
4. The antibody microarray screen according to claim 2, wherein said proteins functionally related with said drug-metabolizing enzyme are selected from the group consisting of mitochondrial proteins, apoptosis-
20 related proteins, anti-oxidant proteins, oxidative stress proteins, and intracellular protein degradation proteins.
5. The antibody microarray screen according to claim 1, wherein said substrate includes a hydrogel (polyarylamide-based) coating.
6. The antibody microarray screen according to claim 1 further
25 comprising labeled secondary immunoglobulins.
7. The antibody microarray screen according to claim 1, wherein said fluids are selected from the group consisting of ascites fluids, hybridoma culture medium, and anti-sera.
8. An antibody microarray screen comprising: a substrate; polyclonal
30 antibodies as purified immunoglobins, wherein said antibodies are spotted on predetermined positions on said substrate; and anti-sera spotted on said predetermined positions on said substrate.
9. The antibody microarray screen according to claim 8, wherein said polyclonal antibodies detect proteins selected from the group consisting of
35 drug-metabolizing enzymes, cytochromes P450, and oxidative stress

proteins.

10. The antibody microarray screen according to claim 8, wherein said substrate includes a hydrogel (polyarylamide) coating.

11. The antibody microarray screen according to claim 8 further including
5 labeled secondary immunoglobins.

12. An antibody microarray screen comprising: a substrate; monoclonal antibodies as purified immunoglobulin, wherein said antibodies are spotted on predetermined positions on said substrate; ascites fluid spotted on said substrate; and hybridoma culture media spotted on said substrate, wherein
10 said ascites fluid and hybridoma culture media are spotted on predetermined positions on said substrate.

13. The antibody microarray screen according to claim 12, wherein said monoclonal antibodies detect proteins selected from the group consisting of drug-metabolizing enzymes, cytochromes P450, and oxidative stress
15 proteins.

14. The antibody microarray screen according to claim 12, wherein said substrate includes a hydrogel (polyarylamide) coating.

15. The antibody microarray screen according to claim 12 further including labeled secondary immunoglobins.

20 16. A method of manufacturing an antibody microarray comprising the step of spotting more than a single concentration of antibodies on a microarray substrate to increase the number of up-regulated protein detection.

17. The method according to claim 16, wherein the antibody
25 concentration is more than 5 $\mu\text{g/ml}$ IgG.

18. An internal control molecule for use in an antibody microarray comprising a protein, wherein said protein is unexpressed in the array sample for normalization of focused (non-global) array data.

19. The internal control molecule according to claim 18, wherein said
30 protein is selected from the group consisting of a Flag protein and a non-mammalian protein.

20. The internal control molecule according to claim 18, wherein the internal control molecule is used to compare the expression ratio of house-

keeping proteins to select housekeeping genes by determining any difference between the control and experimental samples.

21. A method of determining optimal spotting concentrations of IgG comprising the steps of: (a) spotting increasing concentrations of IgG on
5 microarray slides; (b) hybridizing the slides with secondary IgG with a detectable signal; and (c) scanning and quantitating signal strength of each spot and selecting optimal concentrations of IgG.

22. A method to increase a detectable signal with microarray analysis comprising the steps of using an intensive molecular signal, wherein the
10 intensive molecular signal is produced by conjugation of a dye and a reporter molecule to a protein whereby interference of IgG binding to a protein is created.

23. The method according to claim 22, wherein the intensive molecular signal is produced by conjugation of a dye and a reporter molecule to a
15 protein to the extent that interference of Coomassie blue stain binding to the protein is created.

24. The method according to claim 22, wherein the intensive molecular signal is used for antibody microarrays.

25. The method according to claim 22, wherein the intensive molecular
20 signal is used for protein microarrays.

26. A method to increase a detectable signal with microarray analysis comprising the steps of: conjugating of a dye and a reporter molecule to a protein; and creating interference of an IgG molecule binding to the protein.

27. A method of producing antibody microarrays comprising the steps of
25 spotting antibodies for Phase I and II drug metabolizing enzymes and proteins functionally related with the drug-metabolizing enzymes on a microarray substrate.

28. The method according to claim 27, wherein the proteins functionally related with drug-metabolizing enzyme are selected from the group
30 consisting of mitochondrial proteins, apoptosis-related proteins, anti-oxidant proteins, oxidative stress proteins, and intracellular protein degradation proteins.

29. The method according to claim 27, wherein the targeted drug-

metabolizing enzyme antibody microarray includes an internal control to be used for data normalization.

30. The method according to claim 29, wherein the internal control is a Flag protein.